Use of Immunology Techniques in Crime Scene Analysis by Forensic Scientists

Traces of DNA evidence can be magnified with PCR
DNA typing
Identification of unknown substances for the presence of drugs with color indicator tests
Urinalysis for drugs by thin layer chromatography

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ABSTRACT

How do forensic scientists use immunology techniques?

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Lymphoma is the most common type of blood cancer in the United States, primarily of lymphocytes including both B cells and T cells. These cells recognize foreign pathogens and destroy infectious agents and abnormal cells in the body. My summer research experience at the NIH branch of Genetic Disease Research, focused on X-linked lymphoproliferative syndrome. This syndrome severely impairs the immune system's ability to generate serum antibodies while inciting a hyperactive immune response to viral infection. It is a disease characterized by severe immune dysregulation. When individuals with this disease are exposed to Epstein-Barr virus, they exhibit uncontrolled lymphoproliferation. By studying the signal transduction of T-Lymphocytes and how signaling molecules affect the function of T-lymphocytes' response to infection this summer, I learned many laboratory techniques that forensic scientists use while analyzing specimens recovered in criminal investigations, including: (1) PCR which can magnify traces of DNA evidence, (2) DNA typing, (3) drug screening using an ELISA, (4) determination of whether a homicide victim was pregnant, (5) determination of the carrier of a disease in an epidemic. The curricular goal of this unit is to teach students the laboratory techniques employed by forensic scientists and to extend student's knowledge of the immunological basis for how each test functions.

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I. Maryland State Standards:

1.1.1 The student will recognize that real problems have more than one solution and decisions to accept one solution over another are made on the basis of many issues.
1.1.2 The student will modify or affirm scientific ideas according to accumulated evidence.
1.2.2 The student will identify meaningful, answerable, scientific questions.
1.2.3 The student will pose meaningful, answerable, scientific questions.
1.2.6 The student will identify appropriate methods for conducting an investigation (independent and dependent variables, proper controls, repeat trials, appropriate sample size etc.)
1.3.1 The student will develop and demonstrate skills in using lab and field equipment to perform investigative techniques.
1.3.2 The student will recognize safe laboratory procedures.
1.3.3 The student will demonstrate safe handling of the chemicals and materials of science.
1.3.4 The student will learn the use of new instruments and equipment by following instructions in a manual or from oral direction.
1.4.3 The student will use experimental data from various investigators to validate results.
1.4.9 The student will use analyzed data to confirm, modify, or reject a hypothesis.
1.5.5 The student will create and/or interpret graphics. (scale drawings, photographs, digital images, field of view, etc.)
1.5.9 The student will communicate conclusions derived through a synthesis of ideas.
1.7.2 The student will identify and evaluate the impact of scientific ideas and/or advancements in technology and society.
3.2.2 The student will conclude that cells exist within a narrow range of environmental conditions and changes to the environment, either naturally occurring or induced, may cause changes in the metabolic activity of the cell or organism.
3.3.3 The student will explain how a genetic trait is determined by the code in a DNA molecule.

II. Unit Goals:

Students will become aware of immunology concepts used to create the technology Forensic Technicians use in crime labs. Students will investigate a crimescene, examine trace evidence for DNA, identify unknown white powders, and examine urine samples from possible suspects. They will use DNA extraction to obtain DNA from the hair follicle and PCR to magnify the suspects' DNA samples. Students will determine the specific analytic test that should be performed on each item of evidence collected, in order to identify which suspect committed the crime. Choices of evaluation tools will be various tests for drugs or pregnancy, and gel electrophoresis.

After completing this unit, students will be able to distinguish the roles of a Crime Scene Investigator (CSI) and a Forensic Technician. Students will emulate the role of a CSI as they investigate a crime scene. Then, they will evaluate the role of a Forensic Technician as they analyze evidence and determine how to interpret the results. Students will be able to follow the scientific process by formulating a hypothesis, collecting evidence, interpreting results, and drawing a final conclusion that will be used in trial for the criminal case.

III. Student Outcomes:

1. Students will be able to evaluate a crime scene, collect evidence, and determine the specific tests that should be performed to analyze trace evidence collected.

2. Students will demonstrate their understanding of DNA extraction and gel electrophoresis and describe the Forensic applications to proving or disproving a suspect's presence at a crime scene.

3. Students will analyze evidence by using PCR, gel electrophoresis, and Thin Layer Chromatography to identify evidence that may prove or eliminate a suspect's involvement at a crimescene.

4. Students will demonstrate their understanding of the immune system by applying their knowledge of Serology and Forensic Toxicology to the evaluator tool.

IV. Science Background and Teacher Resources:

A PowerPoint presentation is provided to explain the concepts in each lesson. It includes video clips and animations to help the teacher understand and explain the processes explored in this lab investigation. The PowerPoint is broken down into sections with objectives listed for each lesson.

In **Forensic Science,** there is a DNA Unit and a Drug and Toxicology Unit. When designing these lessons and with the teacher's flexibility in mind, the labs can be taught in sequence or within the various units relevant to the lessons topic. For example, Lessons 1-3 can be taught in a DNA unit and lessons 4-8 can be taught during a Drug and Toxicology Unit, Immunology unit in **Biology,** or **Anatomy & Physiology.** Days 1-8 may also be taught in a **Molecular Biology** course. Days 4-8 may also be taught in a **Chemistry** course.

A description of the processes used during these lab investigations and a summary of steps involved will be explained.

DNA Extraction isolates DNA from hair follicles or roots, body fluids, skin, or bone tissue. Steps include the disruption of the cellular membrane, lysis, removal of proteins and contaminants, and recovery of DNA. This investigation uses hair with the root or follicle attached, because hair is more commonly found at a crime scene than fingerprints or body fluids. The hair follicle contains the DNA. Chelex solution is used to prevent heavy metal ions in the cells from interfering with Taq polymerase in the PCR. Place mixtures in vortex or swirl vigorously to ensure that the suspects' hair follicles remain within the Chelex solution. Incubate the contents within the microcentrifuge tube at 56 °C for 5 minutes. Place mixtures in vortex or swirl vigorously again. Incubate at 56 °C for another 10 minutes. Place mixtures in vortex again or swirl vigorously. Incubate in boiling water for 10 minutes to break the cells open. Place mixtures in vortex or swirl vigorously again and then, place mixture on ice for 5 minutes. Spin in microcentrifuge for 5 minutes. Remove 75 uL of supernatant and place in a fresh tube without transferring Chelex beads and without disturbing the pellet at the bottom of the tube (https://www.amherst.edu/system/files/media/1898/2012_Manual_5c_Alu_Lab.pdf).

PCR is used to amplify a segment of DNA. The sample is first heated so the DNA denatures or separates into two pieces of single-stranded DNA. Then, an enzyme called "Taq polymerase" builds two new strands of DNA, using the original strands as templates. This process results in the duplication of the original DNA, with each of the new molecules containing one old and one new strand of DNA. Next, each of

these strands creates two new copies, and so on, and so on. The cycle of denaturing and synthesizing new DNA is repeated as many as 30 or 40 times and leads to more than one billion exact copies of the original DNA segment. The entire process of PCR is completed in a few hours with a Thermocycler, which is programmed to alter the temperature of the reaction every few minutes to allow DNA denaturing and synthesis (http://www.genome.gov/10000207).

> [In this experiment] PCR is used to amplify a nucleotide sequence from chromosome 8 to look for an insertion of a short DNA sequence called Alu within the tissue plasminogen activator (TPA) gene. Over the past 65 million years, the Alu sequence has amplified via an RNA-mediated transposition process to a copy number of about 500,000--comprising an estimated 5% of the human genome. Alu sequences are thought to be derived from the 7SL RNA gene which encodes the RNA component of the signal recognition particle that functions in protein synthesis. Alu elements are approximately 300-bp in length and derive their name from a single recognition site for the endonuclease Alu 1 located near the middle of the Alu sequence.
>
> An estimated 500-2,000 Alu elements are mostly restricted to the human genome. A few of these have inserted recently, within the last one million years, and are not fixed in the human species. One such Alu element, called TPA-25, is found within an intron of the tissue plasminogen activator gene. This insertion is dimorphic, meaning that it is present in some individuals and not in others. PCR can be used to screen individuals for the presence (or absence) of the TPA-25 insertion.
>
> In this experiment, oligonucleotide primers, flanking the insertion site, are used to amplify a 400-bp fragment when TPA-25 is present and a 100-bp fragment when it is absent.
> (http://www.accessexcellence.org/AE/AEPC/DNA/detection.php).

Gel Electrophoresis is used to separate molecules based on their net charge, shape, and size. There are three possible distinguishable genotypes resulting from gel electrophoresis: homozygotes for presence of TPA-25 (400-bp fragment only), homozygous for absence of TPA-25 (100-bp fragment only), and heterozygotes (400-bp and 100-bp fragments).
(http://www.accessexcellence.org/AE/AEPC/DNA/detection.php).

Thin Layer Chromatography in drug screening of urine is used to detect the presence of drugs contained within a urine sample. A "control" urine sample is compared to other urine samples. The other urine samples are labeled unknowns #1-6. These unknown urine samples could possibly contain Caffeine,

dextromethorphan/pseudoephedrine, Dexatrim, Sudafed, or Quinine. The "control" and unknown urine samples are added to a Tox Elut® column filled with diatomaceous Earth to extract possible drugs. After the urine sample moves through the column, the collection beakers contain the extracted fluids. Add three drops of methanol to the collection beakers of the "control" and 6 unknown samples, in order to reconstitute the contents within the collection beakers. Since these samples are left overnight, most of the extracted fluids contained within each beaker evaporated. Then, place 20 uL of the methanol/drug mixture into a capillary tube. Next, release the contents within the capillary tube onto the chromatography plate. The solution is developed by allowing the solvent to move up the plate until it reaches the 9 cm mark. The results are visualized by analyzing their place on the chromatography plate and calculating their Rf value. Substances will be evaluated with a UV light. The possible presence of drugs in each unknown sample is evaluated by calculating the Rf value for each substance isolated by the thin layer chromatography process. The Rf value helps to identify possible drugs.

- **Control** is a 20 mL urine sample that does not contain drugs. All 6 unknown samples are compared to the "control" for drug identification.

- **Developing** is the procedure of placing a silica plate spotted with drugs into a liquid. The liquid is absorbed and moves up the plate.

- **Developing solvent** is the liquid that moves up the silica plate and separates the mixture of drugs into their separate components.

- **Drug Extraction** is the urine sample containing drugs. It is poured through a column of diatomaceous earth. The diatomaceous earth has been buffered to pH = 9. The drugs are later removed by pouring a mixture of dichloromethane, isopropanol, and ethly acetate through the column. The drugs were previously dissolved in this mixture, the urine materials are left behind in the Tox Elut® column filled with diatomaceous Earth.. The drug solution emerging from the column appears as a spot on the chromatography plates.

- **Eluent** is the mixture of dichloroethanene, isopropanol, and ethyl acetate which extracts the drugs from the diatomaceous earth.

- **RF Value** is the ratio of the height of any given drug (measures from the origin) divided by the height of the solvent.

- **Sorbent** is the silica coating on the chromatography plate.

- **Standard** is a pure sample of drugs that is examined in the drug screen. This sample is spotted directly on the chromatography plate without being mixed with urine. The Rf values of drug spots in the "control" and the "standard" should be the same. (Note: This is a solution of the 5 sample drugs and is not combined with the urine sample) In this case, all 5 samples are dissolved according to the enclosed instructions. Then, 10 mL of each sample is then mixed together to make the "combined standard" solution.

- **Unknowns #1-6** are 20 mL samples of urine that contains drugs. Spots of drugs occurring in the 6 unknowns are compared to the "control."
    - The 6 unknowns will all contain a combination of 1 or 2 known drugs for students to identify.
    - Unknowns #1-4 should only contain 1 drug.
    - Unknowns #5-6 may contain a combination of 2 drugs.

- **Visualization:** The process of spraying a developed plate with chemicals that will cause the drugs to react by producing a colorful spot at their location.

- **Urinalysis** is a process that tests urine samples for the presence of specific proteins.

Resources provided:

- Power Points explain the relationship between immunology and forensic lab techniques used in labs by Forensic Technicians or out in the field for Crime scene investigators.
- Power Point slides explaining DNA extraction
- Power Point slides explaining PCR
- Power point slides explaining thin layer chromatography
- Power Point slides explaining immunoassays
- Student Lab sheets

V. Learning Objectives

Lesson 1: Introduction to the Immunology behind presumptive and confirmatory test used by Crime Scene Investigators

- Review how each human has unique characteristic traits found in their DNA as genes.
- Describe how Forensic Technicians uses these principles to identify and exonerate suspects identified by criminal investigations.
- Investigate the crimescene to determine what items should be collected as evidence.
- Use the scientific method to answer these questions:
    - ✓ What would you consider evidence?
    - ✓ How would you collect, store, and document the evidence recovered?
    - ✓ What questions need to be answered to solve the crime?

Lesson 2-5: DNA Extraction and PCR

- Obtain hair samples to extract DNA.
- Perform DNA extraction techniques.
- Perform polymerase chain reaction.
- Analyze samples with gel electrophoresis to see if PCR was successful.

Lesson 6-7: Identification of unknown Substances

- Identify unknown white substances by creating a standard of color identification for known white substances and comparing these standards to the 5 unknowns.
- Perform **presumptive tests or screening tests** at the crime scene and in the lab to further analyze and confirm findings.

Lesson 8-9: Urine analysis thin layer chromatography

- Evaluate the evidence collected to identify the kinds of tests or equipment needed to analyze drugs.
- Analyze evidence, record data, and write conclusions

Lesson 10: Conclusions and Review

- Students will work within groups to discuss experimental results.
- Students will formulate conclusions and identify of the purpose of different analytical tools used by Crime scene Investigators and Forensic Technicians.

VI. Time Required

Lessons 1-10 each require a 45-50 minute class period.
The lessons do not have to be taught in 8 consecutive class periods. If the lessons are related to more than one unit in your curriculum, it may be time efficient to teach Lessons1-5 during one unit and to teach the other lessons at another time. For example, in Forensic Science there is a DNA Unit that occurs before the Drugs, Alcohol, and Forensic Toxicology Unit.

<u>Teacher preparation time:</u>
Lesson 1:

- **10 minutes** is needed to review power point slides and video links.
- **5 minutes** is needed to copy student packet.
- **10 minutes** is needed to set up the crime scene in a section of your classroom that can remain untouched for 2 days.
    - **Set up a cut out of a dead body or chalk drawing of a dead body on the floor with unknown white powders in 5 Petri dish containers around it.**

Lessons 2-5:

- **30 minutes** is needed the afternoon before or the morning of the lab.
- See materials list for day 2 & 3.
- The teacher may decide to make Agarose gels and buffer for running the gel, or the students can make these substances while waiting in between the various stages need for DNA extraction of hair.

Lesson 6-7:

- **30 minutes** is needed the afternoon before or the morning of the lab.
- Use attached labels to set up 8 groups of solutions for the white substance lab.
- See the materials list for Day 6 & 7.


Lesson 8 - 9:

- **30 minutes** is needed the afternoon before or the morning of the lab.
- See the materials list for Day 8 & 9.
- Make 7 set-ups that look like the picture to the right.

Lesson 10:

- No preparation time required.

VII. Materials for a class set of 8 groups

Lessons 2-5 Materials	Quantity	Comments
hairs with roots	3-4 per student	Students will use tweezers or their hands to pull out their own hair. Have 2 students in each group of four pull out their hair.
Tweezers (students may be able to pull out their own hair without it)	16	2 per group. Make sure the root is attached.
Vortex	1	1 container for students to use to fill beakers at their station with all the microcentrifuge tubes containing DNA and DNA primers
56^{0}C water bath	1	
Centrifuge	1	The class can share 1 and take turns. If you have a second one, it decreases the wait time.
Boiling water bath		Use a beaker on a hot plate with a microcentrifuge floater.
DNA ladders • 100 bp ladder (0.13 mg/mL stock in "OG") • OG = orange G loading Dye	2 mL per class	

1x TBE buffer	1000 mL	This should be enough for 3 classes to make gels and to use as gel running bugger. This is a 10% Solution.
P20 & P200 uL micropipettes	8 each	
1.5 mL microcentrifuge tubes	16 per class	
Timer	1	Students can use timers on their phones. Teacher may also set a timer on the board for all students to use.
Micropipette tips For P20 & P200 uL	8 boxes for each	Student groups may also share tip boxes if there are not enough to go around.
Microcentrifuge tube racks	8	
S/M/L Gloves		All gloves and everything that was in contact with ethidium bromide should be placed in the BIOHAZARD bag.
Lab aprons	1 per students	
Goggles	1 per student	
Used tip beaker	8 beakers	
Chelex	4.5 g	Make a 10% Chelex solution with 50 ug/ml protease K.
Protease K	15 mL	
Permanent markers	16	
DNA Up and downstream primers • TPALU 1 primer (stock 25 pmol/ml) • TPALU 2 primer (stock 25 pmol/ml)	1 container of each will be enough for 3 classes	Each should come in its own container of about 5 -10mL.
Staining trays • Use large plastic weigh boats	8 per class	Chemistry large plastic weigh boats can be used instead. Have students place their gels large plastic weigh boats so that they can be disposed of after use.
Paper ethidium bromide sheets	8	Ethidium bromide should not be used by anyone other than the teacher. Teacher will place these sheets on the gel using gloves. The gel trays should be placed on newsprint and all materials used should be disposed of in the Biohazard bag.
Permanent marker	16 per class	
"Ready to go" PCR beads	16 per class	They may come in a package of 50 or 100. Place the ready beads into a 1.5 microcentrifuge tube.
UV transilluminator		Teacher will use this to take pictures of the gels that has been stained with Ethiduim bromide sheets
Loading dye	8 microcentrifuge tubes	Place 1.5 mL of loading dye into one microcentrifuge tube. This should be enough for a lab group to use it 3 or more times.
Biohazard bag	1	1 per class, if you can have them in your classroom
Ice		Make a 2% agarose gel using 5g of agarose and 250mL of TBE solution. Place 30-35 mL
Agarose	8	Solution into each gel tray.

If you are having a hard time finding these supplies in your school, there is a kit by Carolina with some of these reagents, but it may not be enough for more than one class and may contain fewer than 8 class sets. This can all be purchased in a kit by **Human Alu DNA Extraction and Amplification with 0.5-mL Tube Item #211230**

Lessons 6 & 7 Materials	Quantity	Comments
Aspirin	1 box	Use a mortar and pestle to ground the entire box into a powder, and place it in a small bottle container.
Baking Soda Sodium Bicarbonate	1 box	Place it in a small bottle container.
Chalk	1 box	Use a mortar and pestle to ground the entire box into a powder, and place it in a small bottle container.
Contac	1 box	Use a mortar and pestle to ground the entire box into a powder, and place it in a small bottle container.
Cornstarch	1 box	Place it in a small bottle container.
Glucose	1 box	Place it in a small bottle container.
Sudafed	2 box	Use a mortar and pestle to ground the entire box into a powder, and place it in a small bottle container.
Salt	1 box	Place it in a small bottle container.
Flour	1 box	Place it in a small bottle container.
Tylenol	1 box	Use a mortar and pestle to ground the entire box into a powder, and place it in a small bottle container.
Create 5 unknowns from the known you have.		Place it in a small bottle container. One should be Sudafed and one should be Contact. The others can be anything. Suggestions are given in the teacher section.
Iodine		Fill a dropper bottle, and label with the colored labels included in this manual.
Bendict's Solution		Fill a dropper bottle, and label with the colored labels included in this manual.
Methanol		Fill a dropper bottle, and label with the colored labels included in this manual.
Iron (III) Nitrate		Fill a dropper bottle, and label with the colored labels included in this manual.
Hydrochloric Acid		Fill a dropper bottle, and label with the colored labels included in this manual.
Water		Fill a dropper bottle, and label with the colored labels included in this manual.
pH indicators or paper		Fill a dropper bottle, and label with the colored labels included in this manual.
Cell culture plates containing 12 wells		
9 dropper bottles per lab stations	9 per station	
Mortar and pestle	1	 needed to grind the different tablets

This is what each class set should look like.

Labels for dropper bottles

Methanol	Methanol	Methanol	Methanol	Methanol	Methanol	Methanol	Methanol
Hydrochloric Acid 3.0M	Hydrochloric Acid 3.0M	Hydrochloric Acid 3.0M	Hydrochloric Acid 3.0M	Hydrochloric Acid 3.0M	Hydrochloric Acid 3.0M	Hydrochloric Acid 3.0M	Hydrochloric Acid 3.0M
Iron (III) Nitrate 0.1M	Iron (III) Nitrate 0.1M	Iron (III) Nitrate 0.1M	Iron (III) Nitrate 0.1M	Iron (III) Nitrate 0.1M	Iron (III) Nitrate 0.1M	Iron (III) Nitrate 0.1M	Iron (III) Nitrate 0.1M
Universal Indicator	Universal Indicator	Universal Indicator	Universal Indicator	Universal Indicator	Universal Indicator	Universal Indicator	Universal Indicator

CONTACT DEXTROMETHORPHAN/ PSEUDOEPHEDRINE	CAFFEINE	DEXATRIM PHENYLPROPANOLAMINE	SUDAFED PSEUDOEPHEDRINE	QUININE	COMBINED STANDARD	COMBINED CONTROL
UNKNOWN #1	UNKNOWN # 2	UNKNOWN #3	UNKNOWN # 4	UNKNOWN #5	UNKNOWN # 6	

Lessons 8 & 9 Materials	Quantity	Comments
Methanol		Use methanol to add to water to create a 50:50 mixture to dissolve each chemical listed.
Ring stands	6	Attach test tube clamps to ring stands.
Test tube ring stand clamps	6	
Caffeine (caffedrine)	1 open capsule	Add one capsule to100 mL of distilled water and 100mL of methanol.
Contact = Dextromethorphan / pseudoephedrine	1 open capsule	Add one capsule to100 mL of distilled water and 100mL of methanol.

Sudafed = Pseudoephedrine	1 open capsule	Add one capsule to100 mL of distilled water and 100mL of methanol.
Quinine	1 open capsule	Add one capsule to100 mL of distilled water and 100mL of methanol.
Dexatrim	1 open capsule	Add one capsule to100 mL of distilled water and 100mL of methanol.
Urine or synthetic urine can be used	150 mL	Needed per class. You can use urine or purchase synthetic urine.
7 glass bottles with caps	7	
6 ring stands	6	
6 silica gel plates	6	
6 test tube holder clamps to go onto the ring stand	6	
6 capillary tubes	6	
6 tox elute column Filled with diatomaceous Earth	6	
6 spray bottles	6	
6 50 ml beakers	6	
Hair dryer	1	
Eluent solution	2 bottles	
Ninhydrin solution	1 bottle	
Mercury oxide solution		
Rf value cards	6	
Isopropanol		
Simulated urine	1 bottles	1 bottle per class
0,1 N HCl		
Pipettes	6	
Water bath		
Hood		
UV lamps black light	1 or 7	Groups can share one. There are little UV flashlights that you can give each students.
Acetone		
Ethyl acetate		
Ammonium hydroxide		
Methanol		
Chloroplantinic acid		
Sulfuric acid 10% solution		
Ethyl acetate		
25mL Graduated cylinders	6	
16 oz glass Mason jar with a lid	6 or 7	The chromatography plate is inside of the Mason jar.
Capillary tubes	6 per class	
Blow dryer	1	
Combined standard		NO URINE!!! 10mL of each of these samples: Caffeine Contact Sudafed Quinine Dexatrim

Control solution		20 mL of urine or synthetic urine is with 10 mL of the combined standard.
Developing solution		Teacher prepares the developing solvent by adding ethyl acetate, methanol, and concentrated ammonium hydroxide together in a ratio of 17:2:1. a. Add 17 mL ethyl acetate. b. Add 2 mL methanol. c. Add 1 mL concentrated ammonium hydroxide.
Ninhydrin	50 mL	Add 50mL of ninhydrin to 50 mL acetone.
Acetone	50 mL	Add 50 mL of acetone to ninhydrin.
diphenylcarbazone	0.5 g	Add to 25 mL water (short life…place in the refrigerator when mixed).
Mercuric sulfate	0.5 g	Add to 25 mL water (short life…place in the refrigerator when mixed).
Potassium iodide	0.5 g	Add contents of the vials marked chloroplantinic acid to potassium iodide (short life…place in the refrigerator when mixed).

VIII. Teacher Guide

Lesson 1

- **Show students the PowerPoint slides # 1-23.**
- **Ask students to examine the crime scene.**
- **Use the scientific method to answer the following questions:**

1. **What would you consider evidence?**
    - **Bullet casings**
    - **Bullet residues around bullet holes**
    - **DNA samples (Samples can be obtained from hair, skin cells, blood, semen, and saliva)**
    - **Fingerprints, palm prints, and footprints**
    - **Fibers from clothes**
    - **Gunshot residue on hands and clothing**
    - **Gun powder burns**
    - **Insect and mold growth in a body as well as body temperature (to determine time of death)**
    - **Handwriting on a ransom note**
    - **Pattern of gunshot residue spray (This can help determine the distance the shooter was from the victim)**
    - **Presence of chemicals blood spatters**
    - **Residue from accelerants (compounds used to speed up fires set by arson)**
    - **Shoeprints**
    - **Tool marks (marks left on a bullet by a gun when fired)**
2. **How would you collect, store, and document the evidence recovered?**
    - **Write item down on the evidence recovery log.**
    - **Bag items, label date, time, and the person who recoveryed the item.**
3. **What questions need to be answered to solve the crime?**
    - **What should be considered as evidence?**
    - **Who are possible suspects?**
    - **Who committed the crime?**

Lesson 2:

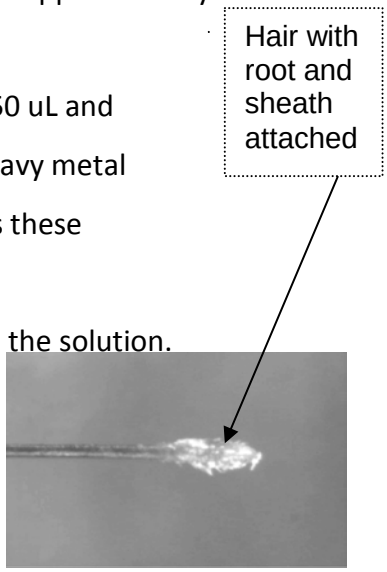
- **Show students slides 24 – 32.**
- **Have students read the introduction to the DNA extraction PCR lab in the student guide pages 1-2.**
- **Have students begin the DNA extraction portion of the lab.**
    - **Students will decide which members of their group will provide hair extraction samples. Two members from each group of 4 will extract hair with follicles on the next school day.**

Lesson 3:

- **Students will put on gloves, goggles, and aprons.**
- **Students will receive a tray containing all the supplies for DNA extraction.**
- **Students will use tweezers to remove hair from two members. Each microcentrifuge tube used for the DNA extraction will receive a number # 1-16 so that students will not be able to know the names of each DNA extraction.**

I. Isolation of Genomic DNA from Hair

1. Obtain 3 to 4 hairs with root and sheath attached. Cut shafts of hair to approximately 2 cm lengths and put in 1.5 ml microcentrifuge tube.
2. Shake Chelex solution. While beads are still re-suspended, remove 150 uL and add to hairs in microcentrifuge tube. Cellular components contain heavy metal ions can interfere with Taq polymerase in PCR reaction. Chelex binds these metal ions and any DNAases that will degrade genomic DNA.
3. Place mixtures in vortex or swirl vigorously. Make sure hairs are in the solution.
4. Incubate at 56 °C for 5 minutes
5. Place mixtures in vortex or swirl vigorously. Make sure hairs are in the solution.
6. Incubate at 56° C for another 10 minutes.
7. Place mixtures in vortex or swirl vigorously. Make sure hairs are still re-suspended.
8. Incubate in boiling water for 10 minutes. This breaks the cell open.
9. Place mixtures in vortex or swirl vigorously and then put on ice for 5 minutes.
10. Spin in microcentrifuge for 5 minutes.
11. Slowly and carefully remove tube. DO NOT disturb the pellet at bottom of tube.
12. Remove 75 uL of supernatant and place in a fresh tube. **DO NOT** transfer any Chelex beads.
13. Label tubes and place on ice or store in freezer until ready to continue.



Make sure the PCR machine has been programmed.

Thermocycler settings: 30 cycles

- **94 C for 1 minute:** The double stranded DNA is denatured to single strands.
- **58 C for 2 minutes**: The primers will attach to specific DNA sequences of single strands.

- **72 C for 2 minutes**: Taq polymerase will add new bases to make the complementary strands.

II. PCR Reaction Set Up and Amplification

1. Use a permanent marker to label a PCR tube supplied by your teacher. It will contain a white bead that contains reagents for the amplification reaction. M**ake sure the bead dissolves when the DNA is added to the tube**.
2. Use the matrix below as a checklist while added reagents to the PCR tube.

Write on the microcentrifuge tube	Hair Cell DNA	Upstream primer	Downstream primer	Total volume
Period #____ 3 Initials of your name	20 uL	2.5 uL	2.5 uL	25 uL

3. Close tube cap tightly and place in block of automated thermal cycler.

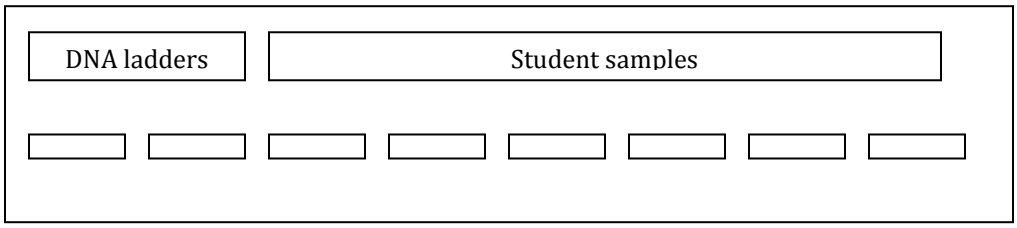
NOTE: Keep previous class sets in the refrigerator or on ice until all your classes are finished. You can load all classes into the Thermocycler at the same time and then you can leave them there all night.

Lesson 4:

- **Students will put on gloves, goggles, and aprons.**
- **Students will receive a tray containing all the supplies for PCR.**
- **Students will receive a tray containing all the supplies for gel electrophoresis.**

IV. Load Gel and Electrophoreses.

1. Remove the gel's comb and the rubber bumpers from the gel box. Place the gel tray into electrophoresis chamber making sure that the wells are facing the negative pole.
2. Add enough electrophoresis buffer (1x TBE) to cover the gel. (Approx. 350 ml)
3. **Add 2 uL of loading dye** to the microcentrifuge tube containing the PCR sample.
4. Slowly mix the solution by pipetting up and down.
   Hint: You will know if you have a clean prep, because mineral oil will not mix with the loading dye. If free of the oil, your DNA sample mixes well with the dye.
5. **Load 20 uL of your PCR reaction** to a sample well in the gel. Note your lane position.
6. **Add 20 uL of DNA ladders** to specified wells in the diagram.



8. **Electrophoreses at 100 volts for about 30 minutes.** Adequate separation occurs when bromophenol blue bands move 30 mm from wells.
9. Turn off power supply, remove gel tray from electrophoresis chamber, and transfer gel to staining tray.
10. Follow protocol for staining with paper ethidium bromide.

Lesson 5:

- **Put on gloves, goggles, and aprons.**
- **Before class, teacher will spread newsprint over an area of the room and place the gels back into the plastic weigh boats. Place the Ethidium bromide paper over each tray.**
- **Show students PowerPoint Slides 33-36.**
  - **These slides will assist students as they complete pages 7-10 of the student packet.**
- **Students can turn in pages 8-10. You can have the students write their data on the large class grid on the board and answer the first few questions as a class. Answers will vary per class.**

1. Calculate the allele frequency for the presence of the Alu insert in the population.
   Note: Humans are diploid and the total number alleles present are 2 x the total population. If there are 30 students in the population that means there are 60 total alleles. **Show your work and circle your answers.**
   Answers will vary per class.
2. Calculate the allele frequency for the absence of the Alu insert in the population. Show your work and circle your answers.
   Answers will vary per class.
3. Give 3 examples of ways crime scene investigators can use PCR to assist in solving a forensic investigation?
   (1) Amplify DNA found in trace samples of hair, saliva, and other body fluids identified at a crime scene.
   (2) Amplify DNA left behind on clothing from cold cases.
   (3) Amplify DNA from bones left behind after a murder.
4. Explain why a forensic scientist **would or would not use** the primers used in this lab procedure in a real forensic case?
   DNA primers attach to the DNA collected and extend them so more copies can be made from the original DNA template found at the crime scene.

5. Is this individual or class evidence? Explain your answer.
   Examples of individual evidence are fingerprints, handwriting, DNA patterns, and sometimes physical matches, However, most evidence is **class evidence**; characteristics common to a group of similar objects, not to one single object.
6. Comment on the Frye Standard; the **"general acceptance"** of this scientific technique. Would your scientific investigation hold up in a court of law if you were asked to take the stand? Please include a discussion of your training, the number of times you have done this technique, your sterile technique (food, gloves, sterilized lab bench), and laboratory procedures you followed (any possible mistakes made during procedure, identify possible steps in procedure that you may have accidentally contaminated your sample)?
   Frye Standard Summary: Defendant James Alphonso Frye was convicted of murder in the second degree and appealed the decision. The issue presented for consideration is that defense counsel offered an expert witness to testify to the result of a systolic blood pressure deception test, a rudimentary precursor to the lie detector, and was denied. They further offered that a test be conducted in the courtroom and were again denied. The prosecution argued that "while the courts will go a long way in admitting expert testimony, deduced from a well-recognized scientific principle or discovery, the thing from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field in which it belongs." The appeals court upheld the assertion of the lower court that the deception test did not meet that criterion (http://biotech.law.lsu.edu/map/TheFryeRule.html).

Steps of Contamination:

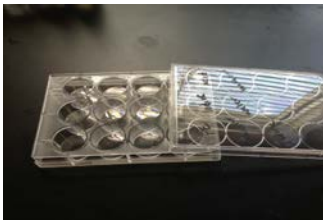
- Not wearing gloves when operating the sample
- Not changing gloves when operating different samples
- Touching surfaces with your gloved hands before operating the sample
- Mislabeling microcentrifuge tubes
- Using micropipette tips for multiple samples… not changing tips between operating PCR reagents

Lesson 6-7:

- Show students PowerPoint slides #37- 43.
- All students should put on gloves, goggles, and lab aprons.
- Use a mortar and pestle to ground up these pills:
    - Aspirin
    - Baking soda (sodium bicarbonate
    - Contac (try to use the white pills only)
    - Sudafed (try to find white pills only use the one that is not over the counter, the over the counter ones have a red coating)
    - Tylenol

- Place these in glass containers or plastic containers with lids.
- Pour these powders into glass containers.
    - Salt
    - Flour
    - Glucose
- Place labels on these containers.
- Place 5 unknown powers in labeled bottles or in the Petri dishes on the forensic crime scene shown on DAY 1.
    - Unknowns should be:
        - Sudafed
        - Contact
        - Glucose
        - Tylenol
        - Flour

- Make sure you have these solutions for each group to use.
        - Iodine
        - Benedicts
        - Methanol
        - Iron (III) Nitrate
        - Hydrochloric Acid
        - Water
        - pH



- Make sure you have students use 2, 12 well, cell culture plates. Students will use a wax pencil to label lids.
- Students will add a small amount of powder to each well.
- Then, add3-5 drops of each solution into the well.
- They are to record the color change, even if the color stays the same
    - Note: If students just write color change or no color change, data cannot be used to identify the unknowns.
    - Even if the color was originally blue and it stayed blue, the student should write blue and not “no color change.”
- Students can use the section for water to drop their pH sticks in and then they do not have to create a new section for reading pH.

Lesson 8:

Teacher preparation before the lesson:

- Show students PowerPoint slides #44 – 54.
- Use a test tube clamp mounted on a ring stand to support each column used. Place a collection beaker under each column. A 50 or 100 mL beaker should work. A maximum of 50 mL of Eluent solution is collected.
- Make 5 different individual "standard" solutions.
    - Dilute the drug samples with 100mL water 100mL methanol mixture. Place each in a glass bottle.
        - Sudafed
        - Contact
        - Caffeine pills (or Caffedrine)
        - Dexatrim
        - Quinine
        - Add 20 mL of urine sample and 50 mL of each drug solution.
        - 6 unknowns need to be made. Place one drug in each sample. You can mix 2 drugs in 2 of the unknowns or leave one unknown without drugs.
        - Label these mixtures unknown # 1-6
- Make a control sample that has 60 mL of urine without any drugs.
- Make a combined standard by placing 10 mL of each of the 5 samples. Add 20 mL of urine to this **combined standard.**
- **Place the water bath inside the fume hood.** The vapors released from these samples are toxic and should only be handled within a fume hood.

During the lesson:

- Have students read the introduction to the lab.
- Teacher will pour 20 mL of the control into the apparatus.
- Have students pour 20 mL of their unknown into their apparatus.
- Show students PowerPoint slides 44-54
- After the solution has passed through the column containing diatomaceous earth, add 20 mL Eluent to the column.

Procedure:

Part I: Extracting the drugs from unknown urine samples

1. Make sure that you have identified your unknown solution. Take a look at the control solution and make sure that you have:
    - **a.** A test tube clamp
    - **b.** Ring stand
    - **c.** 50 or 100 mL beaker
    - **d.** Bottle container with unknown number matching your diatomaceous earth
    - **e.** Graduated cylinder

2. Observe the teacher pour 20mL of the control into the container labeled control. This sample contains no drugs.
3. Why do we need a control?
   Control group in a scientific experiment is a group separated from the rest of the experiment where the independent variable being tested cannot influence the results. This isolates the independent variable's effects on the experiment and can help rule out alternate explanations of the experimental results. Control groups can also be separated into two other types: positive or negative.

   Positive control groups are groups where the conditions of the experiment are set to guarantee a positive result. A positive control group can show that the experiment is functioning properly as planned.

   Negative control groups are groups where the conditions of the experiment are set to cause a negative outcome.
   (http://chemistry.about.com/od/chemistryterminology/a/What-Is-A-Control-Group.htm).

4. What is the difference between a control and an unknown?
The experimenter's goal is to identify what drugs are contained within the 5 unknown urine samples during the experiment. Controls are extremely useful where the experimental conditions are complex and difficult to isolate. Comparing the unknowns to the control helps to identify the distinguish unknowns and isolate its components to containing one or more than one drug in this experimental investigation.

5. Pour 20 mL of the unknown into the column containing diatomaceous earth
6. The drugs that will be identify are:

- Caffeine
- Sudafed
- Quinine
- Dexatrim
- Contact

7. Label a 50 – 100 mL collection beaker with the unknown number.
8. Place the 50-100 mL collection beaker under the column.
9. After the columns have stopped dripping (approximately 10 minutes, unknowns with a combination of drugs may take longer) remove the collection beakers from underneath the columns.

10. Place the collection beakers in a well ventilated area and allow them to evaporate overnight.
    If this is not possible because there is not enough time, place the collection beakers under a hood into the water bath at approximately 65-70°C. Allow the beakers to evaporate until they are dry.

Lesson 9:

Part II: Prepare the developing chamber and the chromatography plate.

1. Take a16 oz glass Mason jar with a lid. The jar will hold the 2" x 4" chromatography plate.
2. The Teacher will prepare the developing solvent by adding ethyl acetate, methanol, and concentrated ammonium hydroxide together in a ratio of 17:2:1.
    - Get a small bottle or a 50 -100 mL beaker.
    - Add 34 mL ethyl acetate.
    - Add 4 mL methanol.
    - Add 2 mL concentrated ammonium hydroxide.
    - This will be sufficient for 5 mL of the developing solvent for 1 class.
    - Double these measurements and there will be 3 mL for each group of 6 set-ups. This will be enough for 3 classes.
3. Add 3-5 mL of the developing solvent to each chromatography chamber.
    The solvent must cover the bottom of the 16 oz Mason jar = Chromatography chamber.
4. Take a 5 x 10 cm chromatography plate. Use a sharp pencil to scribe 2 lines about 1.5 cm apart and down the entire 10cm length of the plate.
5. The 3 tracks will provide room for:
    - One student urine sample
    - One standard
    - One control

    The tracts keep the drug spots separated during development.

Unknown sample
Standard
Control

6. Use the pencil to make a light mark at the 9 cm height on the chromatography plate.
7. Heat the chromatography plate for 3 minutes with a blow dryer on the highest setting (90 °C or 200°C).

Part III: Reconstitution

1. Add 3 drops of methanol in each totally evaporated collection beaker to reconstitute the drug mixture.
2. Swirl the collection beaker slightly to allow the methanol to contact the entire drug residue.

Spot the unknown section of the chromatography plate:

Unknown sample
Standard
Control

3. Place the top of a 20 uL capillary tube into the methanol/drug mixture. Allow the fluid to rise as high as possible in the capillary by normal capillary attraction. **This should be at least 1" in height in the tube**
    - **a.** Bring the tip of the capillary tube into the same spot on the chromatography plate about 1.5-2 cm from the bottom of the plate.
    - **b.** Make small spots the size of a "0."
    - **c.** Allow each spot pressed by the capillary tube to dry before allowing the capillary to redeposit its next spot on the top of the previous spot.

Spot the combined standard section of the chromatography plate:

Unknown sample
Standard
Control

4. Place the top of a 20 uL capillary into the "combined standard" mixture. Allow the fluid to rise as high as possible in the capillary by normal capillary attraction. **This should be at least 1" in height in the tube.**
    - **a.** Bring the tip of the capillary tube into the same spot on the chromatography plate about 1.5-2 cm from the bottom of the plate.
    - **b.** Make small spots the size of a "0."
    - **c.** Allow each spot pressed by the capillary tube to dry before allowing the capillary to redeposit its next spot on the top of the previous spot.

Spot the control section of the chromatography plate:

Unknown sample
Standard
Control

5. Place the top of a 20 uL capillary into the "control" beaker. Allow the fluid to rise as high as possible in the capillary by normal capillary attraction. **This should be at least 1" in height in the tube.**
    - **a.** Bring the tip of the capillary tube into the same spot on the chromatography plate about 1.5-2 cm from the bottom of the plate.
    - **b.** Make small spots the size of a "0."
    - **c.** Allow each spot pressed by the capillary tube to dry before allowing the capillary to redeposit its next spot on the top of the previous spot.

Now, the test chromatography plate contains samples on all three sections.

Unknown sample
Standard
Control

Lesson 10:

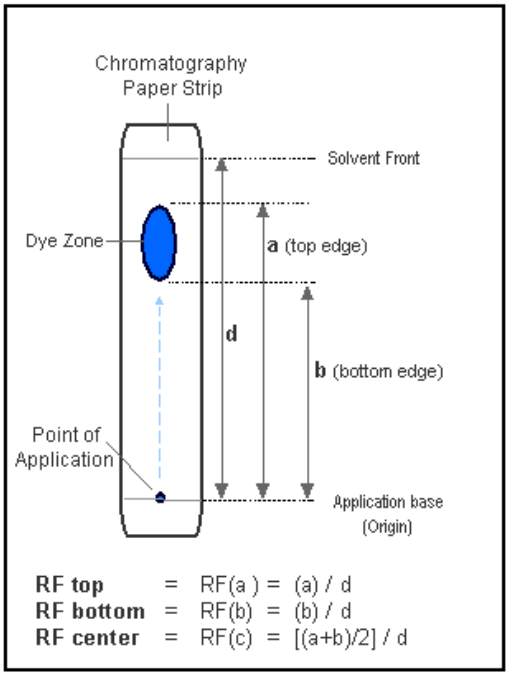
Part IV: Developing

1. Be careful. Only touch the sides of the chromatography plates. Place the chromatography plate in the chamber and replace the cap.
2. Allow the solvent to move up the plate until it reaches the 9 cm mark. **This should take about 20 minutes.**
3. Remove the plate from the chamber.
4. In a well-ventilated area or underneath the fume hood, dry it with the hair dryer at its highest setting.

Part V: Visualization

Solution	RF value	Color	Place on the chromatography plate
Ninhydrin, phenylpropanolamine	0.55		
pseudo ephedrine	0.35	Bright Pink or magenta	Brown spot
Mercury sulfate	N/A	Will turn purple before drying	
Dextromethorphan	0.55	Brown spot	Brown spot
Quinine	0.63	Brown spot Under black light it fluoresces	Brown spot highest on the chromatography plate
Caffeine	0.67	Greenish white	The highest of the three drugs on the chromatography plate
Valium and Librium		Under a black light will fluoresces	

$$\text{Rf Value} = \frac{\text{Distance from Baseline travelled by Solute}}{\text{Distance from Baseline travelled by Solvent (Solvent Front)}}$$



http://www.marz-kreations.com/Chemistry/Chromatography/Dyes/RF-Values.html

Teacher will place these solutions into spray bottles because they are toxic. They are corrosive to the skin, eyes, and mucous membranes.

Ninhydrin,
Iodoplatinate
Diphenylcarbasone

Mercuric sulfate is a poison.

5. Use the spray bottle to place Ninhydrin on the chromatography plate. Be careful. Try to prevent it from running down the plate. Keep the origin end of the plate elevated. Keep spraying the plate until each track on the plate has been spotted.
6. Heat the plate with a hair dryer.
    - Notice the spots of amines forming on top of a pink background.
    - Compare them with the "control" and with the "standard" tracks on the chromatography plate.
7. Place the spotted plate under ultraviolet light for approximately 2 minutes.
    - If you do not have a UV light, a black light, strong sunlight, plant light, or goggle cabinet containing sterilization bulb can be used to enhance the color of the spots on the chromatography plate.
8. Use a spray bottle to place diphenylcarbazone on the plate. Be careful. Try to prevent it from running down the plate. Keep the origin end of the plate elevated. Keep spraying the plate until each track on the plate has been spotted.
9. Heat the plate with a hair dryer.
   Adding additional reagents will destroy the amphetamines, remember to record these numbers.
    - Record the spot position and its color on the picture of the chromatography plate.
    - Compare your spots to the "control" and the "standard."
    - Consult the RF chart to identify the order of the spots in the "standard" and the "control."
10. Spot the plate with mercuric sulfate.
11. Dry the plate lightly with a hair dryer for 3 or 4 minutes. The spots will fade.
12. Place the plate under UV light.
13. Finally, spot the plate lightly with Iodoplatinate solution. The spot will appear immediately in a variety of colors (from yellows to greens to reddish-browns).

Part V: Identification

1. Find each drug on the card of RF values by comparing each spot on the chromatography plate to the "control" and "standard."
2. Calculate the Rf value of each spot on the "control."
    - Sketch the position of the dots on the plate onto the lab handout.
    - Calculate the distance of a spot from the origin. Label this value SD
    - Calculate the distance of the solvent front from the origin. Label this value SF.
    - SD/SF = RF value
    - Carefully compare the Rf values and colors of the "control" to the Rf values and colors listed on the Rf card.
    - Identify all spots on the chromatography chart. If any are missing, ask your teacher to help you.
3. Identify the drugs present in the "unknown" that have been measured.
4. Share your results with the class.
5. Label the spots in all the unknowns on the sample below.
6. Write your calculated Rf on the chart for the class.

Results

Unknown sample
Standard
Control

Sample	SF	SD	RF = SD/SF	Identification of drugs in urine
Unknown #1				
Unknown #2				
Unknown #3				
Unknown #4				
Unknown #5				
Unknown #6				
Unknown #7				

IX. Student Handouts

Name: _____

Instructions:

- **Create a list of items that should be collected as evidence.**

  _____
  _____
  _____

- **How would you collect, store, and document the evidence recovered?**

  _____
  _____
  _____

- **What questions need to be answered to solve the crime?**

  _____
  _____
  _____

Evidence Item Recovery Log Incident #: _____

Item #	Description	Recovered By	Date/Time
	© 2009 – Imprimus Forensic Services, LLC This form can be downloaded at www.forensic-classroom.com	Page _____ of _____	

DNA Fingerprinting and PCR

BACKGROUND

When **Crime Scene Investigators (CSI's**) arrive at the scene many times the sample left behind is not a fingerprint. Most fingerprints are partial prints and are not good enough to match a potential suspect to a crimescene. Blood or hair is readily available at crime scenes, even if found in very small samples. Before CSI's can compare DNA found at the crime scene to the DNA collected from possible suspects, they usually have to use polymerase chain reactions (PCR) to amplify the trace amounts of DNA found at the crime scene.

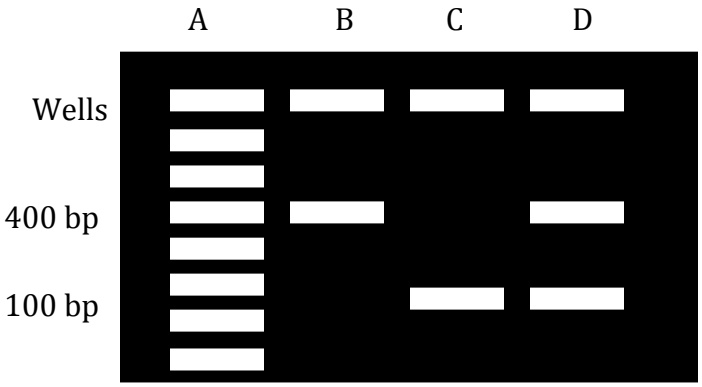
Forensic scientists use a technique developed by molecular geneticist Dr. Ray White in 1980, known as **"DNA fingerprinting."** In 1986, PCR was first used during a criminal case in the United States. The case "Pennsylvania vs. Pestinikas," involved an allegation of a funeral home in Pennsylvania that was accused of switching body parts. (http://www.nfstc.org/pdi/Subject01/pdi_s01_m02_01.htm)

In 1987, DNA fingerprinting was first used by the criminal justice system in England during the **"Footpath Murders."** The police investigating the case were thrilled to hear what Alec Jeffreys was doing with DNA profiling and wanted to solidify their case with this new technology. They asked Alec Jeffery's to examine Richard Buckland's DNA and compare it to the DNA sample left behind in one of the victims clothing. The analysis led to a conclusion the police officers had not anticipated. Instead of corroborating his testimony, the DNA analysis revealed that Richard Buckland was not a match. This evidence was used to exonerate Richard Buckland who made a false confession about raping and murdering two teenage girls after hours of interrogation by Leicester police in England. Analyzing the DNA from the victims clothing also allowed the police to know a little more about their suspect. He was a male with Type A blood, and he also had a rare enzyme found in 10% of the local male population. They eventually did get the correct man, Colin Pitchfork. The wonderful thing about DNA fingerprinting is that it can be used to prove guilt and innocence.
(http://www.trutv.com/library/crime/criminal_mind/forensics/dna/2.html)

CODIS is a DNA database used by police and FBI to identify DNA found at the crime scenes with known suspects. This process relies on probability. The FBI has identified 13 **short tandem repeats (STR)** that are useful to identify or exonerate criminals. The number of repeats within an STR is referred to as an allele of the gene in that individual. The FBI determined that the probability of two unrelated Caucasians having identical alleles at all 13 STRs is approximately 1 in 575 trillion (Reilly, 2001). We will analyze one STR called the **Alu insertion**. When it is **present** it makes an allele that is approximately **400 base pairs** in length. When the Alu **insertion is absent** the length of the DNA fragment is approximately **100 base pairs** in length (http://www.accessexcellence.org/AE/AEPC/DNA/detection.php).

You will perform a PCR on hair samples from possible suspects and compare these DNA samples to the DNA left at the crime scene. It will be your goal to identify which hair sample belongs to the suspect who committed the crime. The results of your DNA profile are crucial.

Remember it is just as important to eliminate a possible suspect as it is to find the correct criminal.

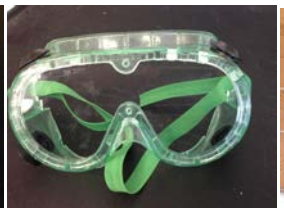


Well A: DNA ladder
Well B: contains DNA from a homozygous +/+ carrier of the Alu trait
Well C: contains DNA from a homozygous -/- carrier of the Alu trait
Well D: contains DNA from a heterozygous +/- carrier of the Alu trait

Chelex solution is used to extract the DNA from these cells. The cells are heated and boiled; this causes them to break open and release DNA molecules. The DNA is spun in a microcentrifuge tube. The **supernatant** containing genomic DNA is placed in a special **microcentrifuge tube** that contains a small white bead. This bead contains the replicating enzyme called **Taq polymerase**, the four deoxynucleotides (Adenine, Cytosine, Guanine, Thymine), and a **cofactor** called magnesium chloride which helps the enzyme Taq polymerase work. After the single stranded primers and some mineral oil are added to prevent the DNA from boiling over, the tube is placed in a temperature cycling machine called a **Thermocycler**. This machine will heat up the DNA to 95 degrees causing it to split into two single strands. When the temperature is lowered to around 65 degrees, the primers border the sides of a targeted sequence of DNA. Finally, the temperature is increased to 72 degrees allowing **Taq polymerase** to build the new DNA. All this happens in just 5 minutes. After 30 cycles, over a million copies of the targeted sequence are produced.

Image taken from: Alu Insertion Polymorphism to study human populations.
http://bioinformatics.dnalc.org/alu/animation/pdf/pv92.pdf

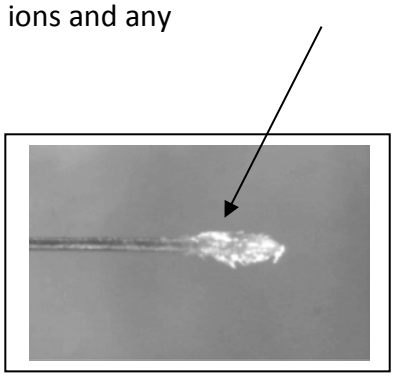
Please wear:


I. Isolation of Genomic DNA from Hair

1. Obtain 3 to 4 hairs with root and sheath attached. Cut shafts of hair to approximately 2 cm lengths, and put in 1.5 ml microcentrifuge tube.
2. Shake Chelex solution. While beads are still re-suspended, remove 150 uL and add two hairs in microcentrifuge tube. Cellular components contain heavy metal ions can interfere with Taq polymerase in PCR reaction. Chelex binds these metal ions and any DNAases that will degrade genomic DNA.
3. Place mixtures in vortex. Make sure hairs are in the solution.
4. Incubate at 56 °C for 5 minutes
5. Place mixtures in vortex. Make sure hairs are in the solution.
6. Incubate at 56° C for another 10 minutes.
7. Place mixtures in vortex. Make sure hairs are still re-suspended.
8. Incubate in boiling water for 10 minutes. This breaks the cell open.
9. Place mixtures in vortex, and then, put on ice for 5 minutes.
10. Spin in microcentrifuge for 5 minutes
11. Slowly and carefully remove tube. DO NOT disturb the pellet at bottom of tube.
12. Remove 75 uL of supernatant and place in a fresh tube. DO NOT transfer any Chelex beads.
13. Label microcentrifuge tubes and place on ice or store in freezer until ready to continue

Hair with root and sheath attached



II. PCR Reaction Set Up and Amplification

1. Use a permanent marker to label a PCR tube supplied by your teacher. It will contain a white bead that contains reagents for the amplification reaction. M**ake sure the bead dissolves when the DNA is added to the tube.**
2. Use the matrix below as a checklist while adding reagents to the PCR tube.

Write on the microcentrifuge tube	Hair Cell DNA	Upstream primer	Downstream primer	Total volume
Period #____ 3 Initials of your name	20 uL	2.5 uL	2.5 uL	25 uL

3. Close tube cap tightly, and place in block of automated thermal cycler.

Thermocycler settings: 30 cycles

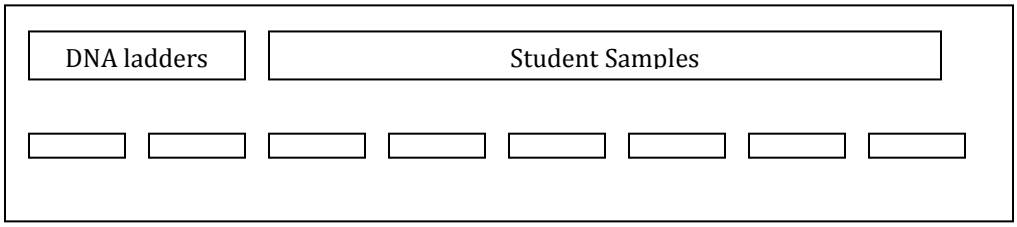
- **94 °C for 1 minute:** The double stranded DNA is denatured to single strands.
- **58 °C for 2 minutes**: The primers attach to specific DNA sequences of single strands.
- **72 °C for 2 minutes**: Taq polymerase add new bases to make the complementary strands.

III. 2.0% Agarose Gel Preparation

1. Carefully, pour agarose solution into the gel casting tray to fill to a depth of 5 mm. The gel should cover about one-third of the height of the comb teeth.
2. Answer questions # 4-8 while you are waiting for your gel to solidify.

IV. Load Gel and Electrophoreses.

1. Remove the gel's comb and the rubber bumpers from the gel box. Place the gel tray into electrophoresis chamber making sure the wells are facing the negative pole.
2. Add enough electrophoresis buffer (1x TBE) to cover the gel. (Approx. 350 ml)
3. **Add 2 uL of loading dye** to the microcentrifuge tube containing the PCR sample.
4. Slowly mix the solution by pipetting up and down.
   Hint: You will know if you have a clean prep, because mineral oil will not mix with the loading dye. If free of the oil, your DNA sample mixes well with the dye.
5. **Load 20 uL of your PCR reaction** to a sample well in the gel. Note your lane position.
6. **Add 20 uL of DNA ladders** to specified wells in the diagram.



8. **Electrophoreses at 100 volts for about 30 minutes.** Adequate separation occurs when bromophenol blue bands move 30 mm from wells.
9. Turn off the power supply, remove gel tray from electrophoresis chamber, and transfer gel to staining tray.
10. Follow protocol for staining with paper ethidium bromide.

Name _____

Results and Discussion

1. After a photograph of your gel has been taken, orient the photograph with the sample wells at the top.
2. Attach photo in space provided and label the lanes 1 through 8, noting which lanes were designated for the ladders and which ones for student samples.

Attach Gel Photo Here

3. Using the DNA ladders as a guide, view the student sample lanes and note any allele bands.

Note: it is possible that the primers may have been amplified themselves creating a primer dimmer.

These are not products from the DNA and should be less than 100 bp in length.

4. Complete the data table below to determine genotype distribution of class.

Student Samples	Homozygous for the insert (+,+)	Heterozygous for the insert (+,-)	Homozygous – no insertion (-,-)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			

Totals			

5. Calculate the allele frequency for the presence of the Alu insert in the population. **Note:** Humans are diploid and the total number alleles present are 2 x the total population. If there are 30 students in the population that means there are 60 total alleles. **Show your work and circle your answers.**

6. Calculate the allele frequency for the absence of the Alu insert in the population. Show your work and circle your answers.

7. Give 3 examples of ways crime scene investigators can use PCR to assist in solving a forensic investigation.

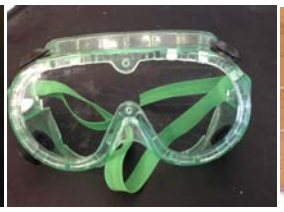
8. Explain why a forensic scientist **would or would not use** the primers used in this lab procedure in a real forensic case.

9. Is this individual or class evidence? Explain your answer.

10. Comment on the Frye Standard, the **"general acceptance"** of this scientific technique. Would your scientific investigation hold up in a court of law if you were asked to take the stand? Please include a discussion of your training, the number of times you have done this technique, your sterile technique (food, gloves, sterilized lab bench, etc.), and laboratory procedures you followed (any possible mistakes made during procedure, identify possible steps in procedure that you may have accidentally contaminated your sample)?

Name: _____
Team Members: _____
Period:___

Please wear:


Procedure:

1. Take a wax pencil and write on the lid of the cell culture container the solutions on the list below. Abbreviations for each solution can be written instead of the entire name.
    - Benedicts Solution
    - $Fe(NO_3)_3$ 3.0 M
    - Hydrochloric acid 3.0 M
    - Iodine
    - Universal Indicator Color
    - Water Solubility
    - pH

2. Place a small scoop, with the spatula, of only one known white substance inside of each of these wells.
   Choose one of the following:
    - Aspirin
    - Baking Soda Sodium Bicarbonate
    - Chalk
    - Contac
    - Cornstarch
    - Glucose
    - Sudafed
    - Salt
    - Flour
    - Tylenol

3. Add 3 drops of each solution to the wells. Make sure you add the correct solution to each well.
    - 3 drops of Benedicts Solution
    - 3 drops of $Fe(NO_3)_3$ 3.0 M
    - 3 drops of Hydrochloric acid 3.0 M
    - 3 drops of Iodine
    - 3 drops of Universal Indicator Color
    - 3 drops of Water
    - Place a pH strip into the well containing water after you have tested the substances solubility.

4. Record the color observed in the chart for the unknown; even if the color stays the same.
5. In order to calculate the pH of the known substance. Place the pH stick into the section of the cell culture plate containing water.

6. Now repeat steps #2→6 until all the known substances are filled out on the table. Remember record the observed color, even if there is no color change.

7. Now repeat steps # 2→ 6 with each unknown. Remember record the observed color onto the chart, even if there is no color change.

White Substances	**Benedicts Solution**	***Fe(NO$_3$)$_3$ 3.0 M***	***Hydrochloric acid 3.0 M***	***Iodine***	***Universal Indicator Color***	***Water Solubility Yes, somewhat, no***	***Methanol Solubility Yes, somewhat, no***	***pH (use the well containing water)***
Aspirin								
Baking Soda Sodium Bicarbonate								
Chalk								
Contac								
Cornstarch								
Glucose								
Sudafed								
Salt								
Flour								
Tylenol								
Crimescene:								
Unknown #1								
Unknown #2								
Unknown #3								
Unknown #4								
Unknown #5								

RESULTS:
Identify each unknown white powder found at the crimescene. Describe the test(s) that allowed you to reach this conclusion.

Unknown #1:

Unknown #2:

Unknown #3:

Unknown #4:

Unknown #5:

What type of test did you perform?

Do you feel your group used great laboratory technique? Explain why or why not?

Explain how color indicator test may be useful to CSI out in the field.

Name: _____
Team Members: _____
Period:___
Please wear:



Urinalysis and Thin Layer Chromatography

Thin Layer Chromatography is used to detect the presence of drugs contained within a urine sample. A "control" urine sample is compared to other urine samples. The other urine samples will be called unknowns #1-6. These unknown urine samples could possibly contain Caffeine, Dextromethorphan/pseudoephedrine, Dexatrim, Sudafed, or Quinine. The "control" and unknown urine samples are added to a Tox Elut® column filled with diatomaceous Earth to extract possible drugs. After the urine sample has moved through the column, the collection beakers will contain the extracted fluids. Three drops of methanol will be added to the collection beakers of the "control" and 6 unknown samples in order to reconstitute the contents within the collection beakers. Since these samples are left overnight, most of the extracted fluids contained within each beaker evaporated. Then, 20 uL of the reconstituted methanol/drug mixture is placed into a capillary tube. The contents within the capillary tube are released onto the chromatography plate. The solution is developed by allowing the solvent to move up the plate until it reaches the 9 cm mark. The results are visualized by analyzing their place on the chromatography plate and calculating their Rf value. Substances are evaluated with a UV light. The possible presence of drugs in each unknown sample is evaluated by calculating the Rf value for each substance isolated by the thin layer chromatography process. The Rf value helps to identify possible drugs.

Vocabulary words you should understand:

- **Control** is a 20 mL urine sample that does not contain drugs. All 6 unknown samples are compared to the "control" for drug identification.
- **Developing** is the procedure of placing a silica plate spotted with drugs into a liquid. The liquid is absorbed and moves up the plate.
- **Developing solvent** is the liquid that moves up the silica plate and separates the mixture of drugs into their separate components.

- **Drug Extraction** is the urine sample containing drugs. It is poured through a column of diatomaceous earth. The diatomaceous earth has been buffered to a pH = 9. The drugs are later removed by pouring a mixture of dichloromethane, isopropanol, and ethyl acetate through the column. The drugs were previously dissolved in this mixture, the urine materials are left behind in the Tox Elut® column filled with diatomaceous Earth. The drug solution emerging from the column will appear as a spot on the chromatography plates.
- **Eluent** is the mixture of dichloroethanene, isopropanol, and ethyl acetate which extracts the drugs from the diatomaceous earth.
- **RF Value** is the ratio of the height of any given drug (measures from the origin) divided by the height of the solvent.
- **Sorbent** is the silica coating on the chromatography plate.
- **Standard** is a pure sample of drugs that is examined in the drug screen. This sample will be spotted directly on the chromatography plate without being mixed with urine. The Rf values of drug spots in the "control" and the "standard" should be the same. (Note: This is a solution of the 5 sample drugs and is not combined with the urine sample.) Then, 10 mL of each sample is mixed together to make the "combined standard" solution.
- **Unknowns #1-6** are 20 mL samples of urine that contain drugs. Spots of drugs occurring in the 6 unknowns are compared to the "control."
    - The 6 unknowns will all contain a combination of 1 or 2 known drugs for students to identify.
    - Unknowns #1-4 should only contain 1 drug.
    - Unknowns #5-6 may contain a combination of 2 drugs.
- **Visualization** is the process of spraying a developed plate with chemicals that will cause the drugs to react by producing a colorful spot at their location.
- **Urinalysis** is a process that tests urine samples for the presence of specific proteins.

Procedure:

Part I: Extracting the drugs from unknown urine samples

1. Make sure that you have identified your unknown solution. Unknown # _____
2. Take a look at the control solution and make sure that you have:
    - A test tube clamp
    - Ring stand
    - 50 or 100 mL beaker
    - Bottle container with unknown number matching your diatomaceous earth
    - Graduated cylinder
3. Observe the teacher pour 20 mL of the control into the container labeled control. This sample contains no drugs.

4. Why do we need a control?

    _____

    _____

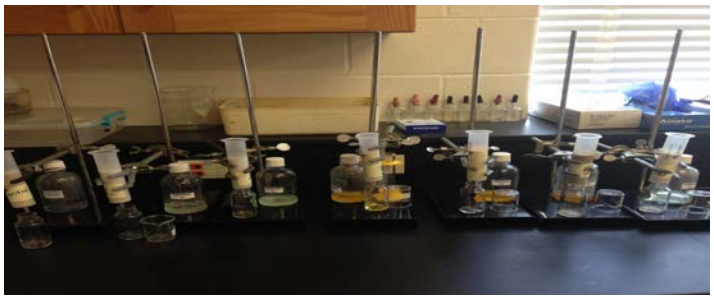
5. What is the difference between a control and an unknown?

    _____

    _____

6. Pour 20 mL of the unknown into the column containing diatomaceous earth.
7. The drugs we are trying to identify are:

    - Caffeine
    - Sudafed
    - Quinine
    - Dexatrim
    - Contact



8. Label a 50 – 100 mL collection beaker with the unknown number.
9. Place the 50-100 mL collection beaker under the column.
10. After the columns have stopped dripping (approximately 10 minutes) remove the collection beakers from underneath the columns.
11. **Place the collection beakers in a well-ventilated area and allow them to evaporate over night. If this is not possible because there is not enough time, place the collection beakers under a fume hood into the water bath at approximately 65°C -70°C. Allow the beakers to evaporate into dryness.**

Part II: Prepare the developing chamber and the chromatography plate.

1. Take a 16 oz glass Mason jar with a lid. The jar will hold the 2" x 4" chromatography plate.
2. Add 3-5 mL of the **developing solvent** to each chromatography chambers.
3. The solvent must cover the bottom of the 16 oz Mason jar = Chromatography chamber.
4. Take a 5 x 10 cm chromatography plate. Use a sharp pencil to scribe 2 lines about 1.5 cm apart and down the entire 10cm length of the plate.
5. The 3 tracks will provide room for:
    - One urine sample
    - One standard
    - One control

    These tracts keep the drug spots separated during development.

Unknown sample
Standard
Control

6. Use the pencil to make a light mark at the 9 cm height on the chromatography plate.
7. Heat the chromatography plate for 3 minutes with a blow dryer on the highest setting (90 °C or 200°C).

Part III: Reconstitution

1. Add 3 drops of methanol in each totally evaporated collection beaker to reconstitute the drug mixture.
2. Swirl the collection beaker slightly to allow the methanol to contact the entire drug residue.

Spot the unknown section of the chromatography plate:

Unknown sample
Standard
Control

3. Place the top of a 20 uL capillary tube into the methanol/drug mixture. Allow the fluid to rise as high as possible in the capillary by normal capillary attraction. **This should be at least 1" in height in the tube.**
    - Bring the tip of the capillary tube into the same spot on the chromatography plate about 1.5-2 cm from the bottom of the plate.
    - Make small spots the size of a "0."
    - Allow each spot pressed by the capillary tube to dry before allowing the capillary to redeposit its next spot on the top of the previous spot.

Spot the combined standard section of the chromatography plate:

Unknown sample
Standard
Control

4. Place the top of a 20 uL capillary into the "combined standard" mixture. Allow the fluid to rise as high as possible in the capillary by normal capillary attraction. **This should be at least 1" in height in the tube.**
    - Bring the tip of the capillary tube into the same spot on the chromatography plate about 1.5-2 cm from the bottom of the plate.
    - Make small spots the size of a "0."
    - Allow each spot pressed by the capillary tube to dry before allowing the capillary to redeposit its next spot on the top of the previous spot.

Spot the control section of the chromatography plate:

Unknown sample
Standard
Control

5. Place the top of a 20 uL capillary into the "control" beaker. Allow the fluid to rise as high as possible in the capillary by normal capillary attraction. **This should be at least 1" in height in the tube.**
    - Bring the tip of the capillary tube into the same spot on the chromatography plate about 1.5-2 cm from the bottom of the plate.
    - Make small spots the size of a "0."
    - Allow each spot pressed by the capillary tube to dry before allowing the capillary to redeposit its next spot on the top of the previous spot.

Now, the test chromatography plate contains samples on all three sections.

Unknown sample
Standard
Control

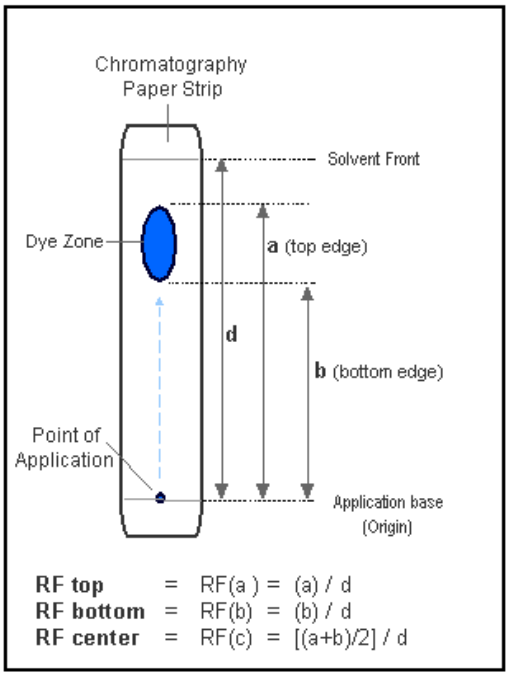
Part IV: Developing

1. Be careful. Only touch the sides of the chromatography plates. Place the chromatography plate in the chamber and replace the cap.
2. Allow the solvent to move up the plate until it reaches the 9 cm mark. **This should take about 20 minutes.**
3. Remove the plate from the chamber.
4. In a well-ventilated area or underneath the fume hood, dry it with the hair dryer at its highest setting.

Part V: Visualization

Solution	RF value	Color	Place on the chromatography plate
Ninhydrin, Phenylpropanolamine	0.55		
Pseudo ephedrine	0.35	Bright Pink or magenta	Brown spot
Mercury sulfate	N/A	Will turn purple before drying	
Dextromethorphan	0.55	Brown spot	Brown spot
Quinine	0.63	Brown spot Under black light it fluoresces	Brown spot highest on the chromatography plate
Caffeine	0.67	Greenish white	The highest of the three drugs on the chromatography plate
Valium and Librium		Under a black light will fluoresces	

$$\text{Rf Value} = \frac{\text{Distance from Baseline travelled by Solute}}{\text{Distance from Baseline travelled by Solvent (Solvent Front)}}$$



http://www.marz-kreations.com/Chemistry/Chromatography/Dyes/RF-Values.html

Teacher will place these solutions into spray bottles, because they are toxic. They are corrosive to the skin, to the eyes, and mucous membranes.

Ninhydrin,
Iodoplatinate
Diphenylcarbasone

Mercuric sulfate is a poison.

5. Use the spray bottle to place Ninhydrin on the chromatography plate. Be careful. Try to prevent it from running down the plate. Keep the origin end of the plate elevated. Keep spraying the plate until each track on the plate has been spotted.
6. Heat the plate with a hair dryer.
    - Notice the spots of amines forming on top of a pink background.
    - Compare them with the "control" and with the "standard" tracks on the chromatography plate.
7. Place the spotted plate under ultraviolet light for approximately 2 minutes.
    - If you do not have a UV light, use a black light, strong sunlight, plant light, or goggle cabinet containing sterilization bulb to enhance the color of the spots on the chromatography plate.
8. Use a spray bottle to place diphenylcarbazone on the plate. Be careful. Try to prevent it from running down the plate. Keep the origin end of the plate elevated. Keep spraying the plate until each track on the plate has been spotted.
9. Heat the plate with a hair dryer.
    - Record the spot position and its color the picture of the chromatography plate.
    - Compare your spots to the "control" and the "standard."
    - Consult the RF chart to identify the order of the spots in the "standard" and the "control."
10. Spot the plate with mercuric sulfate.
11. Dry the plate lightly with a hair dryer for 3 or 4 minutes. The spots will fade. Place the plate under UV light.
12. Finally, spot the plate lightly with Iodoplatinate solution. The spot will appear immediately in a variety of colors (from yellows to greens to reddish-browns).

Part V: Identification

1. Find each drug on the card of RF values. Compare each spot on the chromatography plate to the "control" and "standard."
2. Calculate the Rf value of each spot on the "control."
    a. Sketch the position of the dots on the plate at the end of the procedures.

b. Calculate the distance of a spot from the origin. Label this value SD
c. Calculate the distance of the solvent front from the origin. Label this value SF.
d. SD /SF = RF value
e. Carefully, compare the Rf values and colors of the "control" to the Rf values and colors listed on the Rf card
f. Identify all spots on the chromatography chard. If any are missing, ask your teacher to help you.

3. Identify the drugs present in the "unknown" that you have measured.
4. Share your results with the class.
5. Label the spots of all of the unknowns on the sample below.
6. Write your calculated Rf on the chart for the class.

Results

Unknown sample
Standard
Control

Sample	SF	SD	RF = SD/SF	Identification of drugs in urine
Unknown #1				
Unknown #2				
Unknown #3				
Unknown #4				
Unknown #5				
Unknown #6				
Unknown #7				

X. References

Cannons J.L., Yu L.J., Jankovic D., Crotty S., Horai R., Kirby M., Anderson S., Cheever A. W., Sher A., Schwartzberg P.L. SAP regulates T cell-mediated help for humoral immunity by mechanism distinct from cytokine regulation. *J. Exp. Med.* 203: 1551-65 (2006). http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2118305/

Houck M.M., Siegel J.A.(2006): Fundamentals of Forensic Science 1st Ed

Yin L, Al-Alem U, Liang J, Tong WM, Li C, Badiali M, Médard JJ, Sumegi J, Wang ZQ, Romeo G. Mice deficient in the X-linked lymphoproliferative disease gene sap exhibit increased susceptibility to murine gammaherpesvirus-68 and hypo-gammaglobulinemia. *J. Med. Virol.* 71: 446-55 (2003). http://www.ncbi.nlm.nih.gov/pubmed/12966553

http://www.accessexcellence.org/AE/AEPC/DNA/detection.php

https://www.amherst.edu/system/files/media/1898/2012_Manual_5c_Alu_Lab.pdf

http://biotech.law.lsu.edu/map/TheFryeRule.html

http://biotech.about.com/od/pcr/a/PCRtheory.htm

http://www.carolina.com/pcr-kits/dnalc-human-mitochondrial-dna-extraction-and-amplification-kit-with-05-ml-tubes/211236.pr

http://chemistry.about.com/od/chemistryterminology/a/What-Is-A-Control-Group.htm

http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA460885

http://www.ehow.com/way_5255896_pcr-basics.html

http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab17/lab17.html

http://www.genome.gov/10000207

http://medical-dictionary.thefreedictionary.com

http://www.nfstc.org/pdi/Subject02/pdi_s02_m02_01_b.htm

http://www.nfstc.org/pdi/Subject01/pdi_s01_m02_01.htm

http://www.niaid.nih.gov/topics/immunesystem/Pages/whatIsImmuneSystem.aspx

http://projects.nfstc.org/fse/01/01-05.html

https://www.promega.com/~/media/Files/Resources/Profiles%20In%20DNA/802/Identifying%20and%20Preventing%20DNA%20Contamination%20in%20a%20DNA%20Typing%20Laboratory.ashx

http://serc.carleton.edu/microbelife/research_methods/genomics/dnaext.html

http://serc.carleton.edu/microbelife/research_methods/genomics/dnaext.html
http://www.sog.unc.edu/sites/www.sog.unc.edu/files/Supplemental%20Materials_2012IDXX0007OD.pdf

http://www.sog.unc.edu/sites/www.sog.unc.edu/files/Supplemental%20Materials_2012IDXX0007OD.pdf

http://www.trutv.com/library/crime/criminal_mind/forensics/dna/2.html

http://webcache.googleusercontent.com/search?q=cache:rwn16EXggG8J:media.rsc.org/Modern%2520chemical%2520techniques/MCT5%2520Chromatography.pdf+Royal+society+of+chemistry+chromotography&cd=2&hl=en&ct=clnk&gl=us

http://www.2classnotes.com/digital_notes.asp?p=DNA_Fingerprinting